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Pesticide Biochemistry and Physiology

Volume 62, Issue 2, November 1998, Pages 125-134

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Regular Article

The Mode of Action of Isoxaflutole II. Characterization of the Inhibition of Carrot 4-Hydroxyphenylpyruvate Dioxygenase by the Diketonitrile Derivative of Isoxaflutole^{*1, *2}

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Received 10 April 1998; accepted 31 August 1998. Available online 16 April 2002.

Abstract

Isoxaflutole (5-cyclopropylisoxazol-4-yl 2-mesyl-4-trifluoromethylphenyl ketone) is a novel herbicide for broadleaf and grass weed control in corn and sugarcane which acts by inhibiting the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD). In plants and soil, isoxaflutole is rapidly converted to a diketonitrile derivative (DKN) which is the active herbicide principle. The kinetics of inhibition of carrot HPPD *in vitro* by the DKN showed that it is a potent tight-binding inhibitor (IC_{50} value 4.9 ± 0.2 nM) exhibiting a time-dependent interaction with the enzyme in its ferrous state. Additional investigations provided evidence that the DKN is a competitive inhibitor that rapidly inactivates the enzyme (with a constant rate of association of 0.2 ± 0.004 $\mu\text{M}^{-1}\text{s}^{-1}$) by forming a reversible complex that releases slowly the inhibitor in an unmodified form. The decarboxylation coupled with reduction of molecular oxygen is generally accepted as the first enzymatic event of the HPPD-catalyzed reaction which occurs

as 4-hydroxyphenylpyruvate binds to the internal iron of protein via its ketoacid function. The DKN of isoxaflutole presents a β -(1,3)-diketone moiety, a delocalized π system which can mimic the ketoacid functionality of the substrate and which is also well known for its iron-chelating properties. Since this inhibitor competes with the substrate for binding, it is highly probable that it chelates the ferrous iron in the active site strongly by forming a stable ion-dipole charge transfer complex that resembles the initial substrate-iron complex or an early reaction intermediate. The slow release of the inhibitor in an unmodified form also suggests that the molecular oxygen activation due to ferrous iron generating a powerful oxidant as the inhibitor-enzyme complex forms is probably not occurring.

^{*1} The authors are grateful to their colleagues at the Ongar Research Centre of Rhône-Poulenc Agro involved in the discovery of isoxaflutole, particularly to Tina Woolley for her excellent technical assistance. We are also grateful to Dave Cole for his helpful comments and editing of the manuscript.

^{*2} HPPD, 4-hydroxyphenylpyruvate dioxygenase; HPPA, 4-hydroxyphenylpyruvate; HGA, homogentisate; DKN, diketonitrile of isoxaflutole; NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1, 3-dione.

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